Final Report
Study of the Influence of Environment on the Metabolism of Reptiles and Mammals
Reported 30 October 1953
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Project NR 160-906, Contract N7onr-295, Task Order 38

The form and content of this report differ from that of the semi-annual progress reports. Some matters are discussed here in greater detail than previously, and some, the significance of which is slight, are omitted entirely.

The primary objective of the investigator was to explore the relation between environment and the metabolism of the mammal. Attention has been directed almost exclusively to two specific factors, temperature and altitude. Metabolic manifestations have included: whole animal oxygen consumption, tissue respiration and the intermediary metabolism of fat and protein. To these have been added certain other processes, indirectly associated with metabolism, in particular reproduction.

This report is presented in a series of sections, each dealing with a separate aspect of the entire study.

1. The gas exchange of wild animals native to different environments

The existence of metabolic differences between races of humans or animals has been subjected to two divergent explanations. According to the first of these theories, genetic differences are possible in the sense that one racial strain of the same species may show a higher or lower standard energy production than another irrespective of any immediate environmental considerations. According to the other concept, all quantitative variation in metabolic rate is a reflection of the influence of the local environment upon the individual, and through the individual the group. We have attempted to test these hypotheses in so far as one mammalian species is concerned by selecting representatives of three ecological types, or races, subjecting them to a reasonable period of

adaptation to a common environment, and then measuring the average standard metabolism.

The species is Peromyscus maniculatus, the deer mouse, of enormous range and cosmopolitan habit. The three localities from which the specimens were taken were respectively Berkeley, Tule Lake and White Mountain, all within the state of California. The first principle guiding the selection was geographical remoteness, for each locality is separated from both the others by several hundred miles. It could therefore be presumed than any differences in standard metabolism would be referable to a fixed and stable population. Indeed, that each population represented a distinct strain appeared probable by virtue of deviations in length and color of the fur and the relative shapes and sizes of the ears, tail and head.

The second principle guiding selection of locality pertained to the quantitative differences in two critical environmental factors, temperature and altitude, both of which might conceivably alter individual or group metabolic rates. The Berkeley environment extends from sea level to an altitude of 1,500 feet and the annual temperature range is from approximately 30 to 60 degrees Fahrenheit. We have therefore no serious stress exerted upon the animals with reference to either ambient temperature or oxygen pressure. At Tule Lake the altitude is very close to 4,000 feet. Thus, even though the oxygen pressure is somewhat less than at Berkeley, the difference is not sufficient to constitute a critical factor from the physiological point of view. At the same time, the annual temperature range is much greater. In the winter, the thermometer drops frequently below $0^{\circ}F$, whereas in the summer the temperature approximates that of Berkeley. A temperature stress with no appreciable deficiency in oxygen is thus present. The third group was taken from an elevation of 12,500 to 13,000 feet at the crest of the White Mountain Range, just east of Bishop in the Owens Valley. The chronic hypoxia due to high elevation is severe while the tempera-

ture throughout most of the year ranges from near 40 degrees to below zero Fahrenheit. A very great stress due to both causes is hence unavoidable.

The mice were caught with simple can traps. A total of 9 females and 18 males were caught in the Berkeley area, 20 females and 14 males near Tule Lake, and 11 females and 21 males at White Mountain. After capture the mice were brought into the laboratory in Berkeley and established in regular laboratory cages. All the animals received the same food, the so-called "green diet" used successfully with laboratory rats and mice by the Institute of Experimental Biology on this campus.

Oxygen consumption was used as an index to metabolic rate. Measurements were made by means of a modification of the manometric technique as previously described by Cook, et al. (1950).

Unavoidably in dealing with whole-animal metabolism as described, the degree of physical activity constitutes the principal source of random error. If we are to conserve the semblance of standard metabolism (since, of course, with such organisms we cannot achieve a clinically basal condition), the use of an anaesthetic, or other chemical agent is prohibited. On the other hand, it is legitimate to restrict the scope of muscular action by any means which does not operate upon the biochemical level. We have, therefore, uniformly placed each mouse inside a small cage made of fine gauge wire screen prior to introduction into the respiration chamber. The effect is to restrict wide movements without imposing uncomfortable constriction.

The experimental data in detail showed, as would be anticipated, a tendency for the total oxygen consumption to increase with size of animal and for the oxygen consumption per unit weight to decrease correspondingly. It might therefore have been preferable to express metabolic rates as a function of the body surface, or as a power function of the body weight as has been recommended by Kleiber (1932) and other investigators. On the other hand with

one exception the average weights of all the groups of mice were very close to each other and the spread of individual weights within each group was very narrow. Indeed, preliminary calculations made it clear that very little would be gained by an attempt to eliminate weight differences by mathematical procedures.

The mean values for oxygen consumption are in agreement with the general range of values shown by measurements on wild mice of comparable weight as reported by Morrison (1948) and Pearson (1948). However, of special importance to the present study are the distinct differences of metabolic rate seen in the three populations when measured under standard conditions.

The populations from Tule Lake and Berkeley show a mean oxygen consumption higher than that found among the White Mountain mice (see table 1). The differences are highly significant since the t values, or the Critical Ratios of the means, range from 3.1 to 6.5. At the same time, there is no significant difference in weight between populations of a given sex with the exception of the females from Berkeley. The latter group, to be sure, differs widely in metabolic rate from the females from White Mountain but the statistical significance of this difference loses its force since the Berkeley females are considerably smaller than those from White Mountain, and one might easily attribute the metabolic difference to the disparity in size. That the size here is the responsible factor is borne out by the fact that the oxygen consumption per unit weight of the Berkeley females is also much in excess of that of the Tule Lake females.

With respect to the six groups as a whole, however, the data indicate that the White Mountain mice tend to show a lower metabolic rate than those from either of the other two localities.

That metabolism may vary among species of small mammals is indicated by the work of Morrison (1948), Ratfield (1939), Benedict and Lee (1936), Lee (1939, 1940) and Benedict and Petrik (1930). Pearson (1947, 1948), having

noted that between species of the same weight range there may be widely varying rates of metabolism, took the view that the metabolic rate is not closely associated with taxonomic, ecological or anatomical factors. Such inter-specific variations have, however, been attributed to the characteristics of the diet by Wu and Chen (1929) and to genetic influence on body size by Kleiber and Cole (1950).

The present study, which uses statistically adequate numbers of mice, indicates clearly that metabolic variations may exist within one species when the latter is segregated according to geographical races, even though the latter fall within the same weight range. The lower metabolic rate found in White Mountain mice, as pointed out previously, cannot be ascribed to difference in weight between the specimens from this race and those from the races inhabiting the areas of Berkeley or Tule Lake. Furthermore the racial distinction persists even after all three groups have been maintained under conditions as nearly identical as possible for a period exceeding two months.

Several factors might be invoked as explanation for the lower standard metabolism of the White Mountain population. One of these might pertain to the average, or over-all level of physical activity displayed by the three groups. The influence on metabolism of consistent variation in activity during the determinations of oxygen consumption was emphasized by Benedict and Riddle (1927) with steers and by Benedict and MacLeod (1929) with rats. These investigators found that a minor degree of activity is in general without effect upon metabolism, but repeated, intermittent activity may induce an average increase of 15 to 20 per cent increase in metabolic level. More recently Morrison (1948) has shown that a difference of 70 to 90 per cent exists between the minimum and maximum oxygen consumption of Peromyscus. The minimum occurs during the daylight hours, during repose and the maximum at night, when the animals normally become most active. In our experiments the

random fluctuations in oxygen consumption induced by rapid, casual and unpredictable changes in physical activity were cancelled out by the use of long exposure periods in the respiration chamber, restriction of movement during the experiment, and the employment of a reasonably large number of individual mice. Secular, or diurnal shifts, in exercise and hence in metabolic rate were obviated by measuring the gas exchange always during the same six-hour period, from 9 A.M. to 3 P.M. With respect to the three strains of Peromyscus, no systematic and sustained difference in activity level could ever be detected.

In the case of any study with wild animals the ages of the individuals are not known. That the basal metabolism of humans decreases with age has been pointed out by many investigators, for instance Harris and Benedict (1919). A similar fall in metabolism with advancing age has been noted with rats by Mitchell and Carman (1926) and Davis (1937). Despite a contrary opinion expressed by Benedict and MacLeod (1929), the view has been generally accepted that there is a reduction in metabolism as the animal grows older. Whether the age factor was of significance in this investigation of Peromyscus cannot be stated with certainty, for the possibility is present that the population samples may have been drawn in such a way as to show unintentional bias with respect to age. On the other hand, no such effect is readily detectable, and variation due to this factor was minimized by employing animals which were clearly adults as determined by hair color and body weight.

Other potentially disturbing factors are season, environmental temperature at the time of measurement, and sex. These can be ruled out since the temperature surrounding all the groups was identical from the time that they were brought into the laboratory, since the oxygen consumptions were all determined at the same time of year, and since the sexes were segregated as shown in the tables.

There remain, as possible determining factors, the features of the

environments from which the various animals were drawn. When these are examined there is found one strikingly distinct environmental condition which pertains specifically to the group native to the White Mountain area. All the White Mountain animals used in this study were trapped at an altitude of from 12,500 to 13,000 feet, indicating that in their native state they were the only group of the three investigated which had been subjected to conditions of hypoxia. The central questions which arise in this connection are whether the low metabolic rate demonstrated by this race at low altitude is characteristic of the race at high altitude, and if the low metabolism is an adaptation calculated to increase resistance to reduced oxygen tensions. It must be conceded that no categorical answer can be given at present to these questions.

Numerous investigations have been carried out, relative to the possible effect of hypoxia on the over-all oxygen consumption of mammals. Many of the available reports indicate that the oxygen consumption remains unchanged during adaptation. Recent evidence supporting this point of view has been brought out by Lipin and Whitehorn (1950), Houston (1946), Houston and Riley (1947) and McCrery et al. (1943). On the other hand Blood et al. (1948), Kottke et al. (1948) and Sundstroem and Michaels (1942) have shown that one of the first responses made by mammals when they are subjected to hypoxic conditions is a reduction of body temperature and respiratory gas exchange. The problem, therefore, must be regarded as still awaiting final solution. In the present instance the data clearly indicate that White Mountain Peromyscus maniculatus, when studied at low altitude, show a low metabolic rate in comparison with other strains of the same species.

We can furnish no unequivocal clue to the physiological mechanism which is responsible for the observed low metabolic level. Nevertheless at least two reasonable hypotheses may be mentioned.

In 1932 Kleiber disoussed what he called the "specific insulation" of

an animal, by which he referred to the length and thickness of the hair in the pelage. The White Mountain mice have fur which is definitely longer and thicker than that characterizing the Tule Lake or the Berkeley animals. The heat loss of this race is consequently less than that of the other two races under comparable conditions of cold and hence they would require less oxygen to maintain body temperature. This feature might be beneficial under the extreme winter conditions at the summit of the White Mountain range. That this is a reasonable supposition would be implied by the work of Sumner (1932) who cited many cases in which there is a natural selection of races of Peromyscus by virtue of a distinctive pelage closely adapted to the native environment.

In opposition to this theory, however, stands the fact that the mice from Tule Lake showed the same metabolic level as those from Berkeley, but a higher level than those from White Mountain. The winter temperatures at Tule Lake are of the same general degree of severity as those at White Mountain although the season of cold weather does not last as long. Hence to ascribe the metabolic difference to the temperature factor leads to a serious inconsistency.

The alternative suggestion is that altitude is the critical factor. If so then we must assume that the low oxygen tension characterizing the racial environment has in some manner induced a reduction of metabolic rate and that the difference between strains reflects a biochemical and tissue modification. Concerning the nature of such a modification we have as yet no information.

Table 1

Average values for oxygen consumption of three strains of Peromyscus, Berkeley, Tule Lake and White Mountain. The sexes are shown separately.

	Males			Females		
	White Mt.	Tule Lake	Berk-eley	White Mt.	Tule Lake	Berk-eley
O_2 Consumption, per Animal per hour	64.8	75.6	73.2	64.6	72.8	71.1
O_2 Consumption, per gram per hour	2.49	2.85	2.89	2.58	2.95	3.33

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2. The gas exchange of laboratory mice with reference to temperature and altitude

Male mice of three different strains were exposed for periods of 70 to 90 days to ambient temperatures of 4° and 35° C. Periodically the animals were returned to the comfort zone (23° C) for approximately 6 hours for the purpose of measuring metabolic rate. Parallel measurements were made in all cases on groups of similar animals which had been kept for equivalent periods at 23°. Oxygen consumption and carbon dioxide production were determined by the modified manometric technique described in Section 1 of this report. Different strains of mice were used in order to improve the statistical validity of the entire experiment and to detect possible variations on the intraspecific level. The essential data are given in Table 2.

With the Swiss and the C_3H strains the animals altered their energy intake in the direction of the environmental stress and emerged with body weights similar to those of the control groups. That the energy intake was thus altered was shown by the fact that the mice in the cold ate nearly twice as much as the controls, but those in the heat ate only slightly more than half as much. Nevertheless the oxygen consumption and carbon dioxide production in the exposed animals showed no significant difference from that determined with the controls. The gaseous metabolism therefore followed body mass, or size, rather than total food consumption.

This conclusion appeared to be borne out by the anomalous behavior of the A-strain. The animals of this type which were exposed to a temperature of 4°C for 88 days consumed an average of 4.3 grams of food per mouse per day, as compared with 3.5 grams for the control group. On the other hand the mean body weight of the low-temperature animals was 22.7 grams as opposed to 27.1 grams for the controls. The higher rate of gas exchange of the former mice thus appears to be associated with the smaller body size.

On the whole these experiments, plus others which are not reported in detail, appear to indicate that adaptation to low or high temperature is not accompanied by any marked change in whole-body gas exchange, except that which is imposed by nutritional and size considerations.

In order to assess the possible influence of altitude on the whole-animal metabolism a set of measurements was carried out using a small decompression chamber. Two lots of from 15 to 20 male Swiss mice were established in cages and fed standard laboratory diet in the usual way. One lot was retained under ordinary laboratory conditions. The other was placed in the decompression chamber, and except for feeding every third day, was permitted to remain continuously at a simulated altitude of 16,000 feet.

At the end of thirty days, and at intervals of a few days each thereafter, the mice in the chamber were removed and their oxygen consumption measured by the manometric method. Simultaneous measurements were made with the control group. For each group a total of 182 determinations was thus obtained. The mean value for the mice under decompression was 3.23 ml. O_2 per gram mouse per hour, and that for the control animals 3.35 ml. O_2. The value of t, or the critical Ratio of the means was 1.875, which, for 182 degrees of freedom, places the probability of a real difference close to the 5 percent level. There is some indication, therefore that low oxygen pressure may be associated with a reduction in over-all metabolic rate.

TABLE 2

Strain and number of determinations	Exposure	Mean body weight in grams	O_2 consump. in ml/gm/hr.	CO_2 prod. in ml/gm/hr.
Swiss (48)	4°C-73 days	31.8	2.74	1.94
Swiss (48)	Control	33.1	2.60	1.98
Swiss (18)	35°-71 days	29.1	2.49	1.91
Swiss (48)	Control	32.5	2.60	1.98
C_3 H (42)	4°-80 days	32.6	2.65	1.81
C_3 H (36)	Control	32.1	2.57	1.72
A-strain (48)	4°-88 days	22.7	3.98	2.14
A-strain (48)	Control	27.1	2.90	1.92

3. Tissue respiration with reference to temperature and altitude.

The magnitude of the metabolic gas exchange of whole animals is subject to modification from numerous sources: body size, nutritional state, muscular activity and others. A much better base of stability is attained by the individual tissues, of which the liver furnishes a convenient and representative example. Consequently the attempt has been made to discover any alterations in respiratory metabolism of liver slices in vitro, using the mouse as the test animal. In some cases diaphragm was also employed.

Measurements have been confined to oxygen consumption and have been made by the conventional manometric techniques with the Warburg apparatus.

The effect of temperature stress has been studied with several series of animals. For various reasons most of the early attempts suffered from technical inadequacies. Hence only the final results are here described.

Two strains of mice have been employed up to the present time, the Swiss and A strains. With each type two tissues were investigated; liver, and striated muscle from the diaphragm. Each tissue was taken from animals which had been exposed to extensive periods at ambient temperatures of respectively 4° and 33° C. For each experimental group the tissue oxygen consumption was measured in a parallel group of animals maintained at approximately 23° C. Each group, experimental or control, contained approximately 20 animals. The data are summarized in table 3.

It is evident from an inspection of the table that there is very little effect induced by exposures to heat or cold up to 120 days. In only two cases (Swiss, diaphragm 120 days at 4° and A-strain, liver, 13 days at 4°) were the differences between the experimental and control sets of animals statistically significant. Since those two cases showed opposite effects to be induced by cold it is clear that little reliance can be placed on them. There is some possibility that a transitory increase in oxygen consumption

may occur during the period of adaptation to cold, that is during the first few days of exposure, but this point has not as yet been settled. No permanent alteration appears to take place.

In view of the apparant difference in whole-animal metabolism between the wild mice taken from White Mountair and those living at sea level we wished to check the possible influence of altitude, or hypoxic stress on tissue respiration. Accordingly a group of male Swiss mice were placed in a decompression chamber at a simulated altitude of 16,000 feet, and a control group was kept at sea level. After an exposure period of 20 days representatives of both groups were sacrificed at intervals and the Q_{O2} of the livers determined manometrically. 21 control animals were used and 23 experimentals. The mean Q_{O2} for the former was 8.45 and for the latter 7.74. The value of t for the two means was 5.17, a level which is highly significant.

It will be remembered that Peromyocus from White Mountain showed a lower whole animal oxygen consumption than did representatives of the same species from Berkeley and Tule Lake. Laboratory, Swiss-strain, mice yielded a slightly lower value for whole animal consumption if exposed to a chamber altitude of 16,000 feet. Liver respiration under similar conditions showed a definite reduction. From these experiments therefore there is some reason to suspect that hypoxic stress may in some manner lower metabolic rate.

TABLE 3

Strain	No. of days of exposure	Mean		t value
		Control	Expt.	
Swiss	13 days at 4° (liver)	5.63	5.92	0.9354
Swiss	40 days at 4° (liver)	6.87	6.59	0.7179
Swiss	120 days at 4° (liver)	6.46	6.52	0.1714
Swiss	40 days at 4° (diaphragm)	7.50	7.30	0.2816
Swiss	120 days at 4° (diaphragm)	5.45	3.68	3.2777
Swiss	40 days at 4° (heart)	2.33	2.39	0.2142
Swiss	40 days at 33° (liver)	7.66	7.53	0.3227
Swiss	40 days at 33° (diaphragm)	6.02	6.44	0.6461
A	13 days at 4° (liver)	5.20	5.94	3.5238
A	40 days at 4° (liver)	6.35	6.64	0.7250
A	40 days at 4° (diaphragm)	6.88	6.60	0.3783
A	40 days at 33° (liver)	6.01	6.16	0.6000

4. Fat storage as a function of environmental temperature.

In addition to energy production we regarded it as desirable to study the influence of temperature stress upon the material metabolism of certain body components, in particular fat and protein. With respect to fat it was deemed worth while to reinvestigate the quantity and distribution of the body fat of the laboratory rat and mouse subsequent to exposure for several weeks to high and low ambient temperatures.

In the experiments involving rats the lipid was extracted separately from the skin, liver and carcass of each animal. This was accomplished by digestion and saponification of the tissue with an alcoholic, 30 per cent solution of potassium hydroxide, acidification with sulfuric acid, and subsequent extraction with petroleum ether at 25° C. The ethereal solution was washed repeatedly with distilled water. Finally the petroleum ether was removed by distillation on a steam table and a constant weight of the lipid was obtained. No attempt was made to dehydrate; the lipid was kept in a cool, dark drawer and allowed to come into equilibrium with the moisture in the air. The data presented for mice were secured by the same general method, with the exception that each animal was digested in toto and no attempt was made to partition the fat from various regions of the body.

Melting point determinations of the rat lipids were secured by attaching a capillary tube containing a representative sample of lipid to the bulb of a thermometer and gently heating the bulb in acid. Despite the criticisms of Schmidt and Nielsen, Acta Physiol. Scand. 12:110 (1946) the most reproducible results were obtained by observing the clear point and expressing this as the melting point of the lipid. It is recognized that these values are from 1 to 3 degrees higher than the true melting point.

The individual experimental procedures and results are herewith described.

Mice, series A. Table 4 gives the data obtained from an initial series of white mice, "A strain". All of the animals were maintained on stock diet and were divided into three groups of six individuals each. The first group was kept for two months at a temperature of 23° C and is regarded as the control group. The second and third groups were similarly exposed to temperatures of 35° and 4° respectively. At the end of the two month period all the animals were sacrificed and the total lipid determined, the values in this case being expressed as tripalmitin equivalents per 100 grams animal. A laboratory accident destroyed some of the animals; hence we have data for six mice at 23°, four at 4° and two at 35°. The results indicate a fat content greatest in the animals kept at 35°, intermediate in those at 23° and least in those at 4°.

Mice, series B. The data obtained from female, A-strain, white mice are shown in table 5. For this test we used two groups of animals, four kept at 4° C and five kept at 35° C. The exposure period was six months. The data are expressed as total lipid and as lipid per gram animal. The entire carcass of each animal was digested and analyzed as a whole. There is a clear difference between the two groups; the animals exposed to heat contain considerably more total lipid than those exposed to cold.

Rats, series A. Table 6 includes data relative to lipid distribution in three groups of female rats (Long-Evans strain). The first group was exposed to a temperature of 4° C (5 rats), the second to 23° C (6 rats), and the third to 35° C (5 rats). The exposure time was four months. The results indicate that the rats kept at the lowest temperature contained the least total fat, whereas there was little difference between those kept at 23° and 35°. For the carcass, skin, and liver lipid the data are similar: little difference between those kept at 23° and 35° with a considerably smaller

quantity found in the rats exposed to low temperature.

The determination of melting points was performed on three samples from each lipid extraction. Furthermore two determinations were secured from each sample. Only small variations were detected in extractions which had been kept for a week or more in the laboratory. This was interpreted as evidence that there was no general rancidity in the extracted products with corresponding formation of peroxides and changes in the saturation of the lipid.

Table 6 contains the data pertaining to melting point determinations. The carcass lipid of the cold group had a melting point slightly below that of the control group and the latter had one just below that of the group kept in the heat. However, there is no significant difference in the melting points of the lipids from the skin. The liver lipids from the group at high temperature appeared to have a slightly lower melting point than those from the groups at lower temperatures. These differences are all of very low magnitude and there is serious question whether they possess any real significance.

Rats, series B. Three groups of six male rats each were exposed for five months to the same temperatures as were used for the preceding sets of animals. The same portions of the animal body were analyzed for total lipid and for melting point as with the rates in series A. The results are shown in table 6. The total fat, carcass fat and skin fat were at their maximum in the rats exposed to heat, and at their minimum in those exposed to cold, with intermediate values in those maintained at 23° C. The level of liver fat was almost identical in all three groups. With respect to carcass fat there is an apparent correspondence between the melting point and the temperature of exposure, although the numerical differences between the mean values are not large. The trend of melting point with temperature is sub-

stantially the same in both the female and the male rats, insofar as carcass fat is concerned. The values for skin and liver lipid are of the same order of magnitude in series B as in series A. Furthermore, as in the latter series, there is no detectable influence on the part of temperature.

TABLE 4

Lipid content of individual male mice exposed for two months to three different temperatures. Results are in tripalmitin equivalents per 100 grams animal.

	0.34		0.37		0.47
	0.30		0.40		0.51
	0.41		0.41		
	0.40		0.54	mean	0.49
	0.36				
	0.40	mean	0.43		
mean	0.37				

TABLE 5

Total lipid content of individual female mice exposed for six months to temperatures of 4° C and 35° C.

4°		35°		
grams lipid	grams lipid per gram animal	grams lipid	grams lipid per gram animal	
2.772	0.1130	2.977	0.1400	
1.941	0.0880	3.435	0.1520	
2.343	0.0920	3.717	0.1620	
1.890	0.0890	3.037	0.1420	
		4.260	0.1810	
2.238	0.0955	3.485	0.1554	mean

TABLE 6

Percent of fat and its melting point in the whole animal, carcass, skin and liver. Exposure to three temperatures for four months. Each group consisted of either five or six rats. The change in weight is given as percent increase or decrease throughout the entire exposure period. The food consumption is expressed as average grams of food per gram rat per day. The fat is expressed as percentage by weight at the end of the exposure period. The melting point is in degrees centigrade.

Temp.	Food	Weight Change	Percent of fat in whole animal				Melting point		
			carcass	skin	liver		carcass	skin	liver
			A FEMALE RATS						
4°	.0445	∔3.37	10.60	8.04	20.0	5.36	27	29	42
23°	.0531	∔5.38	12.70	9.78	25.5	4.58	30	31	45
35°	.0971	- 2.22	6.85	4.84	14.7	2.66	32	29	48
			B MALE RATS						
4°	.0619	∔66.3	9.73	6.67	20.6	4.28	25	25	44
23°	.0762	∔62.4	7.00	4.49	15.2	4.50	29	27	44
35°	.1070	∔52.5	5.18	3.27	11.7	4.17	32	25	42

The observations just described, pertaining to four completely different series of rats and mice agree in demonstrating that the total fat content of the animals varies inversely with the ambient temperature to which they are exposed for long periods. Less clearly the same trend is present in the carcass and the skin lipids and there seems to be no association between temperature and liver lipids. The explanation is reasonably straightforward. The fat derived from the diet is put to two primary uses: it may

be oxidized to supply energy and it may be stored subcutaneously for insulation. In small mammals kept at a high environmental temperature there is little if any need for insulation; hence no unusual storage is necessary in the skin. On the other hand the energy requirements are low. The fat obtained with the food must therefore be stored, even though it is not immediately required either for insulation or calories. At a low temperature lipids are essential for both these purposes. There arises thus a conflict of interest, regardless of the over-all magnitude of the lipid intake. From the results it is clear that the need for calories through combustion of fat is more pressing than the need for insulation. Perhaps it might also be inferred that the destruction of fat, with an accelerated production of heat, is more effective for maintaining body temperature than the conservation of fat, with an augmented retention of heat. This point of view is reinforced by consideration of the total dietary intake of the experimental rats. The male rats at 4° consumed an average of 106.8 mg. of food per gram rat per day, those at 23° consumed 76.2 mg. and those at 35° consumed 61.9 mg. The consumption by the females was similar. It is evident, therefore, that even in the face of almost double the fat and carbohydrate intake the rats exposed to cold store less and burn relatively more of the available lipid.

The data for the melting points of lipids in the carcass and skin of male and female rats are paradoxical. They show a moderate tendency for the melting point in the carcass to increase with rising ambient temperature, but no such tendency in the skin. If the melting point of the fat is determined in any degree by the actual temperature of the body at the region where the fat is deposited then one would anticipate a variation of melting point with temperature of the skin, which is readily influenced by the external temperature, but not in the carcass where temperature is maintained at relative constancy regardless of external conditions. Similarly no effect

can be detected in our animals which could be referred either to the presence or absence of sweat glands, or to chronic vascular states, such as dilation or constriction of small vessels in the skin. It is, of course, possible that the rat and the mouse are of such small size that the temperature differences with depth do not correspond to those present in larger animals such as were investigated by Henriques and Hansen, and Schmidt and Nielsen. If this is true then our experiments should be repeated using animals of greater size.

5. Nitrogen metabolism

The following experiments were designed to (a) gain detailed information on the nitrogen metabolism of animals exposed to heat or cold and to (b) determine the influence of the testes and the thyroid on nitrogen metabolism as modified by temperature. This information was correlated with changes in body weight. For the purposes of the experiment groups of adult male rats (Long Evans) were subjected to three experimental temperatures; 4° C, 23° C, 35° C and periodic urine collections were made during a 50 day interval. At all times the animals were fed a high caloric diet, rich in vitamins and minerals, but low in protein (5% casein). The urine of the experimental animals was analyzed for total nitrogen, urea, ammonia, allantoin, creatinine, creatine, and uric acid.

a. Normal Rats: Those animals exposed to cold consumed more food than the control. The hot group consumed less food than the control. All animals continued to gain weight, but those animals exposed to cold exhibited smaller growth rates.

Cold animals showed (see Table 7) increased total nitrogen excretion, increased urea excretion, increased allantoin excretion, increased creatinine excretion, increased creatine excretion, and an unchanged ammonia

and uric acid excretion. This was accomplished by excreting a urine concehtrated in urea and creatine, but dilute in ammonia, allantoin, creatinine, and uric acid.

Hot animals showed decreased total nitrogen excretion, decreased urea excretion, increased ammonia excretion, and an unchanged allantoin, creatinine, creatine, and uric acid excretion. This was accomplished by excreting a urine dilute in erea, allantoin, creatinine, creatine, and uric acid, but equally concentrated in ammonia.

b. Castrated Rats: Those animals exposed to cold consumed more food than the control. The hot group consumed less food than the control. All animals continued to gain weight at similar rates.

Cold animals showed increased total nitrogen excretion, increased urea excretion, increased allantoin excretion, decreased ammonia excretion, and an unchanged creatinine and uric acid excretion. This was accomplished by excreting a urine concentrated in urea and allantoin, dilute in ammonia, and unchanged in creatinine and uric acid.

Hot animals showed a decrease in total nitrogen excretion, a decrease in urea excretion, a decreased ammonia excretion, increased creatinine excretion and an unchanged allantoin and uric acid excretion. This was accomplished by excreting a urine dilute in urea and ammonia concentrated in creatinine, and unchanged in allantoin or uric acid. The castrated groups showed no creatine excretion.

c. Thyroidectomized Rats: Those animals exposed to heat consumed less food than the control. All animals lost weight at similar rates.

Hot animals showed a decreased total nitrogen excretion, a decreased urea excretion, a decreased creatinine excretion, a decreased uric acid excretion, and an unchanged allantoin, ammonia, and creatin excretion. This was accomplished by excreting a urine dilute in urea and uric acid, concentrated

in ammonia and allantoin, and unchanged in creatinine and creatine.

As manifested by changes in food consumption it is clear that exposure to heat or cold does alter the energy requirements of the rat. When plotted against temperature, nitrogen consumption and total nitrogen excretion show a typical linear regression. With respect to the various nitrogenous constituents of the urine, it is to be expected that the quantitative amounts excreted when plotted against temperature will show simular regressions provided that they are also contingent on an energy or nutritional level. Thus, from the data it is evident that urea, allantoin, and creatine excretion are dependent on the food consumption at a given temperature. Ammonia excretion on the other hand shows a complex relationship evidently due to the influence of more than one factor. Creatinine and uric acid show only slight modifications due to temperature stress.

There appear to be definite shifts in water metabolism as judged by the data on urine concentrations and shifts in acid base balance as judged by ammonia excretion.

We can conclude that adaptation to heat or cold is contingent only on the satisfaction of the energy requirements of the rat and not dependent on the qualitative or quantitative excretion of substances in the urine, nor is it dependent on the endogenous nitrogen metabolism of the rat.

The changes in the metabolic pattern do not appear to be entirely due to hormonal alterations. Changes in muscle tone could satisfactorily account for changes observed in the cold group. Decrease in total activity appears to be responsible for changes observed in the hot group.

TABLE 7

Temperature and nitrogen metabolism. All figures are in milligrams of nitrogen per rat per day. N- normal rats, C- castrated rats, T- thyroidectomized rats. Temperatures are those at which the animals were exposed.

	Nitrogen consumed in the diet			Total nitrogen excreted			Urea nitrogen excreted		
	N	C	T	N	C	T	N	C	T
4°	1.05	1.10	--	1.24	1.30	--	0.95	1.10	--
23°	0.65	0.54	0.49	0.59	0.69	0.50	0.49	0.55	0.41
35°	0.50	0.35	0.31	0.42	0.47	0.33	0.35	0.38	0.23

	Ammonia excreted			Creatine excreted			Creatinine Excreted		
	N	C	T	N	C	T	N	C	T
4°	0.040	0.024	--	0.016	0.000	--	0.014	0.014	--
23°	0.040	0.077	0.068	0.008	0.000	0.003	0.011	0.012	0.006
35°	0.058	0.054	0.069	0.007	0.000	0.002	0.012	0.012	0.005

	Uric acid excreted			Allantoin excreted		
	N	C	T	N	C	T
4°	.0031	.0025	--	0.062	0.057	--
23°	.0032	.0022	.0008	0.048	0.038	0.018
35°	.0032	.0022	.0007	0.044	0.035	0.018

6. Effects of environment on reproductive physiology

The program of work consisted of several parts, each essential in coordinating various phases of knowledge for a more complete understanding of the effects of the environment on reproductive performance:

a. Consistent live trapping in natural habitats with particular emphasis at 12,000 feet and 4,000 feet.

b. Establishment of outdoor cages at each of these two elevations in order to more closely study breeding activity of local and transposed animals.

c. Short term experimental projects in small outdoor cages designed to test various hypotheses of reproductive regulation.

d. Correlation of information from each of these three sources to establish certain principles.

White Mountain station was selected as the site for these experiments since changes in elevation of 8,000 feet occur within a fairly small area. There are several mammals which range between the extremes of these elevations and several others which are restricted to definite elevation boundaries. The disposition of the latter, however, seem more determined by the nature of the habitat than by any direct relationship to elevation per se. Peromyscus maniculatus was chosen as the experimental animal because their numbers are continuous between the floor of the Owens Valley at 4,200 feet and the top of White Mountain at 14,200 feet. Since the whole population within this area is in reproductive continuity all individuals must be of the same species. It is quite unlikely, therefore, that any differences in reproductive performance at different elevations would be of a genetic difference since there is not sufficient linear distance to act as a substantial barrier to intercommunication. Movement is sufficiently small for any one individual within its lifetime, however, that any animal wild trapped had almost certainly spent its life within a few hundred yards of the spot. Any effect that the environment

might have on the reproductive performance of these animals would show up differently in different areas since it would be able to act in any area throughout the life time of any animal.

Live trapping was conducted at regular intervals throughout the year near Bishop and whenever possible nearthe upper laboratory on White Mountain at 12,000 feet. Trapping was done by can and multiple catch trap, both of which proved very successful. Althcugh a few animals could be found under a wide range of habitats, concentrations could be found only in more specialized habitats. These were along permanent waterways in the Owens Valley and among vegetated rock piles atop the mountain.

From the first trappings on White Mountain in early July, 1952, all mature animals were in full breeding condition until late September of that year. On the basis of pregnant females taken that summer and from a count of placental scars in others, the average calculated litter size for that elevation is 7.6, while the litter size for the Bishop area is only 5.3. Numbers of corpora lutea of pregnancy in all examined were found to be identical with the number of implantations. Concurrent trapping at intermediate elevations between the two principal sites showed a cline wherein animals entered reproductive quiescence at successively later dates with decreasing altitude. About October first nearly all Peromyscus m. above 12,000 feet had become quiescent while by mid-October those at 10,000 feet were just going out of condition. All of these animals remained quiescent until about mid-March and remained in full condition through May with litters beginning to appear only toward the end of this period. At 4,200 feet at least some animals could be found in full condition through the entire fall and winter. A transition to partial quiescence, however, could be seen over a short period of time in December when no litters were observed. All animals below about 8,000 feet returned to full breeding condition in early February, produced litters over

a period of one month, and then went out of condition in mid-March with no further reproductive activity through May.

This becomes a most perplexing problem if we try to reconcile these two different patterns of reproduction in terms of the differences in environmental factors. Why, for instance, should low altitude animals enter quiescence in March while existing in a habitat rich in food, shelter and of fine weather conditions, while those animals above 10,000 feet should remain in full capacity at temperatures averaging around 12° F., with no new plant growth and poor shelter being flooded frequently by melting snow?

A cursory study of endocrine glands during April and May showed large thymus glands and fairly large adrenals in the quiescent low altitude animals indicative of secretion of few cortical steroids and hence of little physiological stress. The animals on White Mountain, however, showed much smaller thymus and adrenal glands indicative of a higher secretion of cortical steroids and probably a more sustained condition of stress. The stress as experienced normally on White Mountain is not sufficient to suppress reproductive development -- at least during these months. The answer to quiescence of Bishop animals may lie in the pituitary with effects reflected in gonadotropic hormones but not in cortical steroids.

The advent of high littering in Bishop during February boosted the population density considerably such that in April, young taken in traps equaled the number of adults. This density is much greater than any local area found on White Mountain above 10,000 feet. It would seem that there might be some casual relationship between the condition of the reproductive organs and the population density of that area. Factors of stress may still be important in contributing to the quiescent state under very severe environmental conditions but certainly is not the whole answer to seasonal breeding.

Data accumulated incidental to other experiments also tend to support

the hypotheses that a high population density inhibits reproduction. Two types of cages were built so that marked animals could be transposed from one site to another and kept under observation with control animals trapped locally kept in other similar cages. Two large 3 x 6 foot cages were placed at each principal site and made to conform as nearly as possible with natural habitat conditions. Each cage was stocked with 18 animals. Although all animals were in full breeding condition when introduced in August, 1952, all went out of breeding condition in a few weeks, contrary to the state of the wild animals living just outside the cages. A number of smaller cages containing only one male and one female apiece showed much closer conformity to the wild population. The numbers in two of the smaller cages increased to five or more during the winter while decimating factors reduced the numbers in three of the larger cages to a relatively low number. In February the cages with a low population came into breeding condition while those of higher density showed only a slight improvement in reproductive development or none at all. The numbers in the one large cage still carrying a full complement were then reduced to 12; within two weeks 8 of the 12 had come into full reproductive condition. It was interesting to note that in the smaller cages the reproductive behavior of the animals corresponded closely with that of the local wild population and not necessarily with that of the population from which those animals had been originally taken. Whatever the factors regulating breeding, they seem to affect all members of the species alike regardless of the area from which they were taken. The differences noted in reproductive performance at the same time of the year between the different altitudes must then be due to some physical factor of the environment or to some factor affecting the animal phychologically.

A number of short term experiments were set up from time to time to test as many of these physical factors as possible. Temperature experiments

were run using a refrigerator and a heated box, photoperiod experiments were tried subjecting the animals to different light conditions, and food experiments were run trying wide varieties and combinations of food. None of these showed any significant correlation with breeding performance which might answer the situation met in the field. Results of the photoperiod experiments indicate, however, that light does have some effect and that under a number of adverse conditions as is usually met in late fall, the added effect of short days may be sufficient to put the animal into reproductive quiescence. This same short photoperiod experienced under more favorable environmental conditions might not be sufficient to lower the animal below the threshold point.

Another experiment was set up in March using a number of small outdoor cages. Each of the 8 cages represented a different combination of number of animals and number of each sex. About half of these were in full breeding condition when entered while the rest were quiescent. Any effect of population density or sex ratio on reproduction should be seen in results of this experiment. No pronounced effect took place in any cage for two months at which time the quiescent animals approached full breeding status. Litters occurred only in cages provided with no more than two males regardless of the number of females in the cage. All animals under experimentation have shown remarkable conformity in physiological response when subjected to similar conditions; individual breeding records, however, show considerably more variation. For this reason much more emphasis is placed on the condition of the gonads in interpreting experiments and observations than on any breeding record. To a large extent this eliminates the problems introduced by eccentric individuals who conform physiologically with the rest but have varying interests in the opposite sex.